

Fig. 1

28S

18S



HiB5

HiB5pUbi1zNBN22

HiB5pUbi1zGDNF14

Fig. 2

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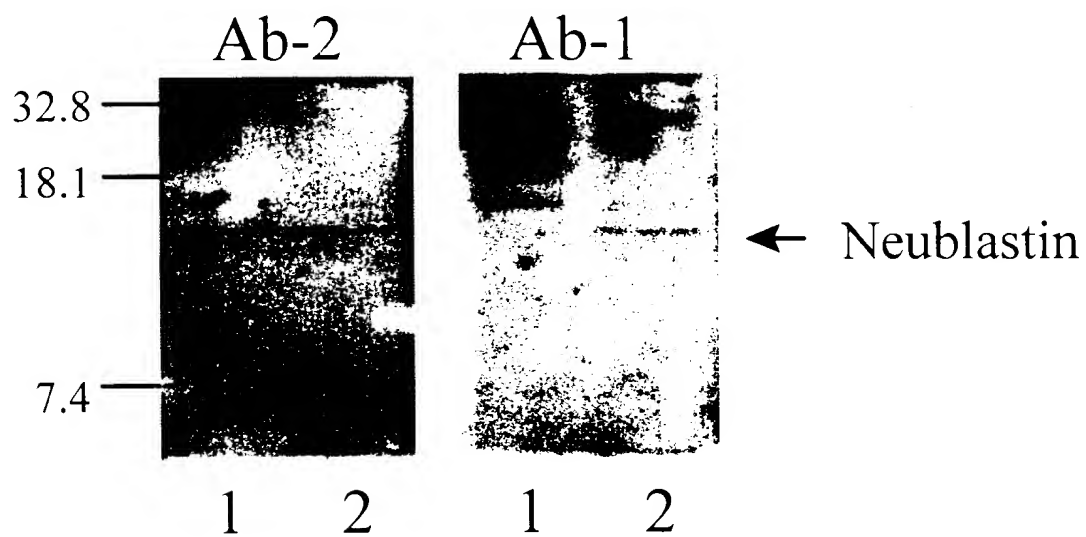
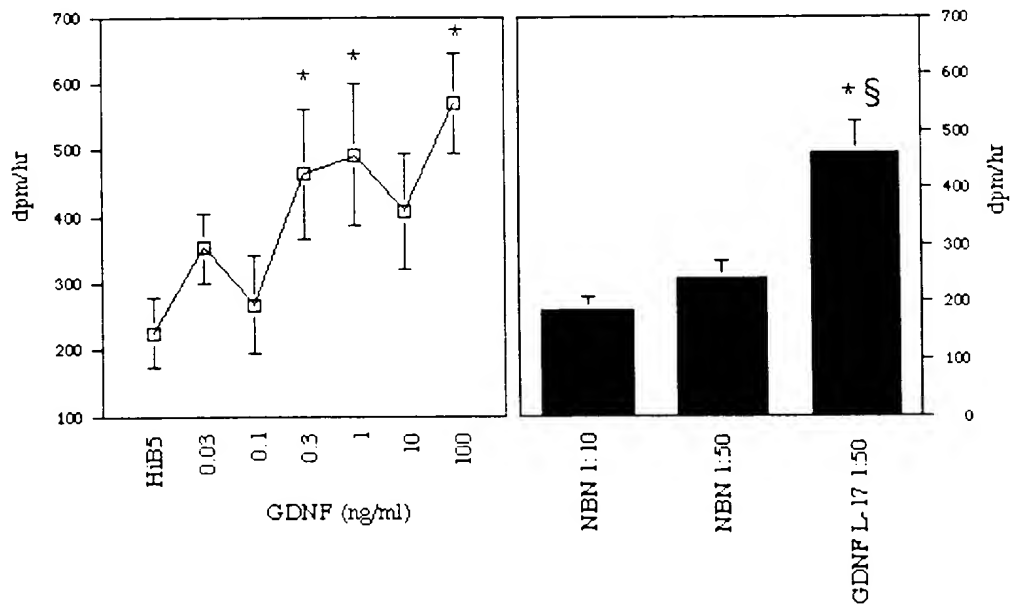
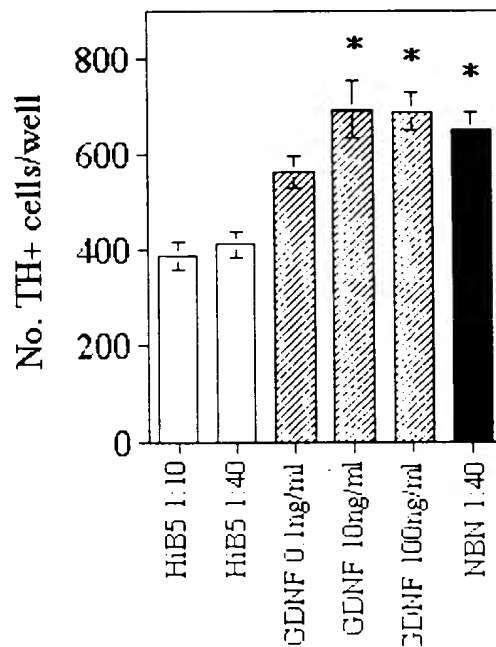


Fig. 3

NBN1 ChAT bioassay



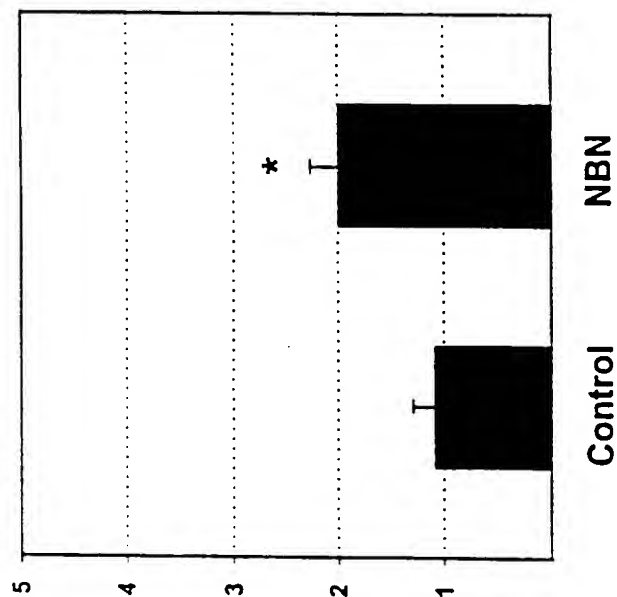
TH+ cell number at DIV 7



Figs. 4A, 4B and 4C

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Dopamine (pmol/ml) - day 12



Dopamine (pmol/ml) - day 21

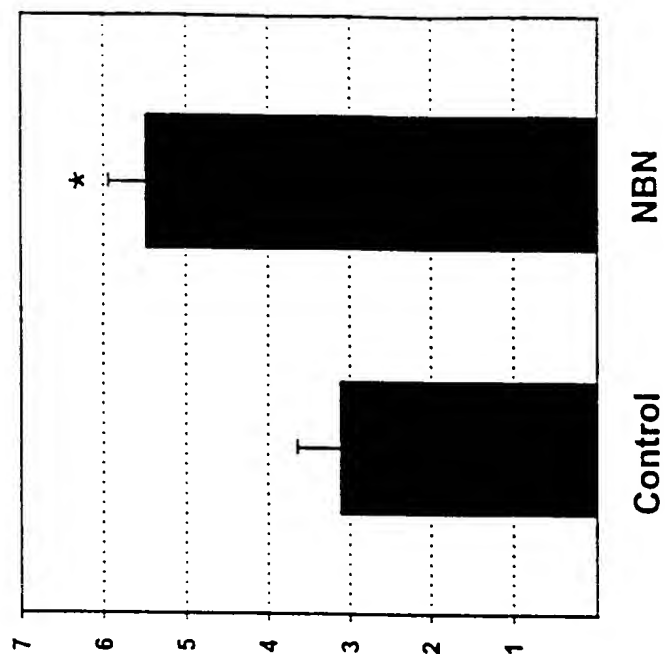


Fig. 5A and 5B

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TH-ir cells per culture

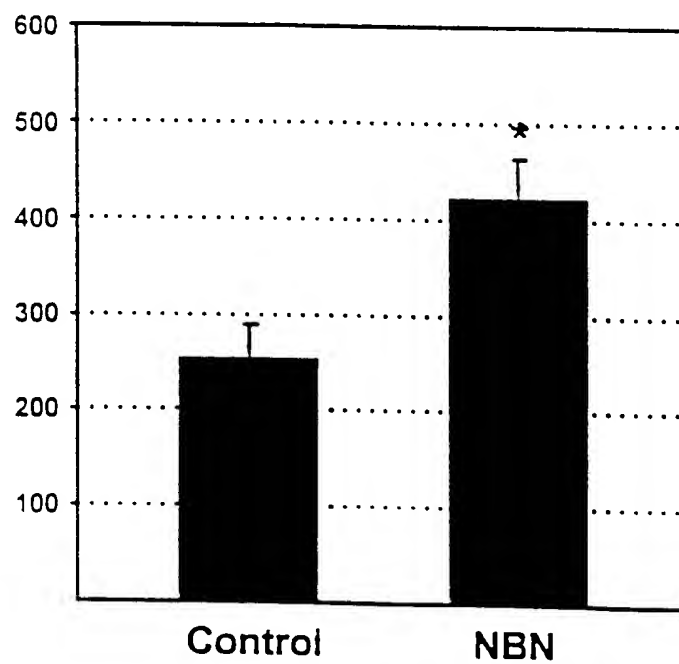


Fig. 5C

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%FG lesion/intact

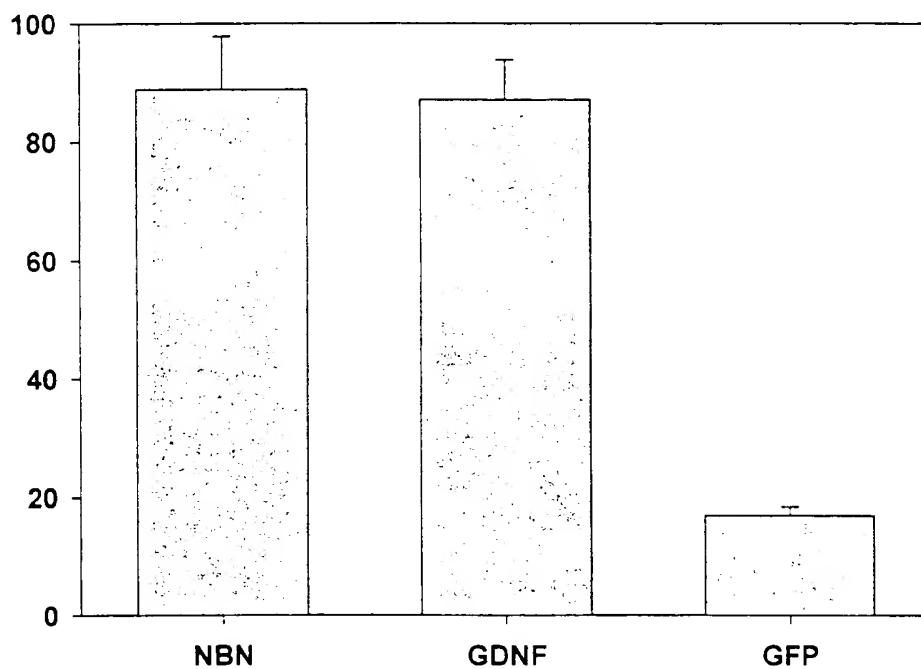
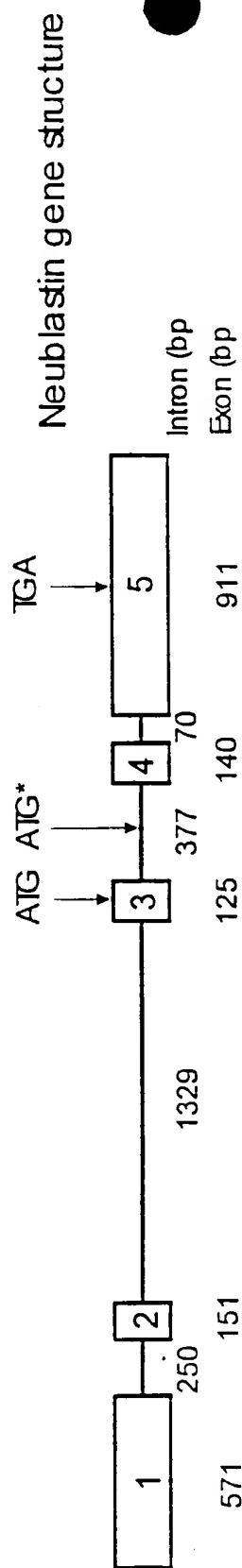
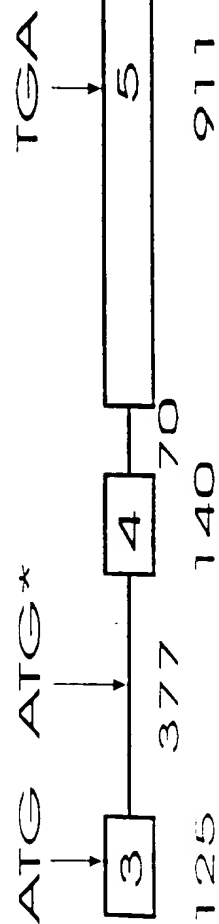


Fig. 6

8114



Seq. Id number 8



290 bp fragment identified in TBLASTN search

Genomic neublastin sequence amplified

NBN primers

Seq. Id number 17
Seq. Id number 18
Seq. Id number 21
Seq. Id number 22
Seq. Id number 23
Seq. Id number 24
Seq. Id number 25
Seq. Id number 26

Fig. 7

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Alignment of Neublabin primers used in Rapid-Screen with
homologous regions in other GDNF ligands

5' -C CTG GCC AGC CTA CTG GG-3'	SEQ ID No 17
G CTG GCC CGG CTG CAG GG	persephin
G CTG CGA CGA CTG CGC CA	neurturin
A TTG AAA AAC TTA TCC AG	GDNF

5' -AA GGA GAC CGC	TTC GTA GCG-3'	SEQ ID No 18
TA GGC CAC GTC	GGT GTA GCG	persephin
AA GGA CAC CTC GTC	CTC GTA GGC	neurturin
AA CGA CAG GTC ATC	ATC AAA GGC	GDNF

conserved nucleotides shown in **bold**

Fig. 8

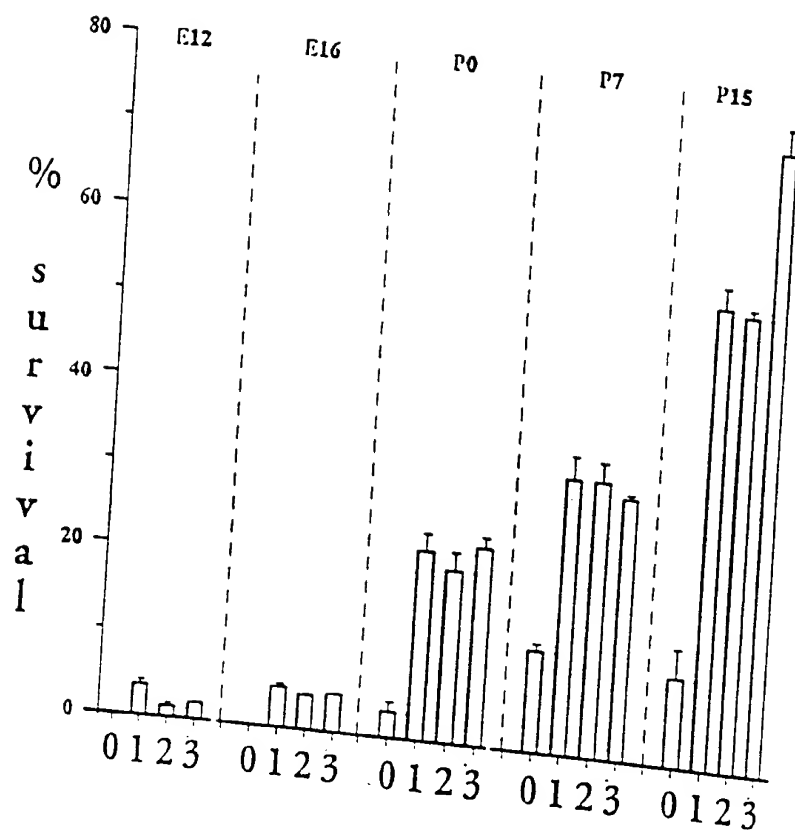
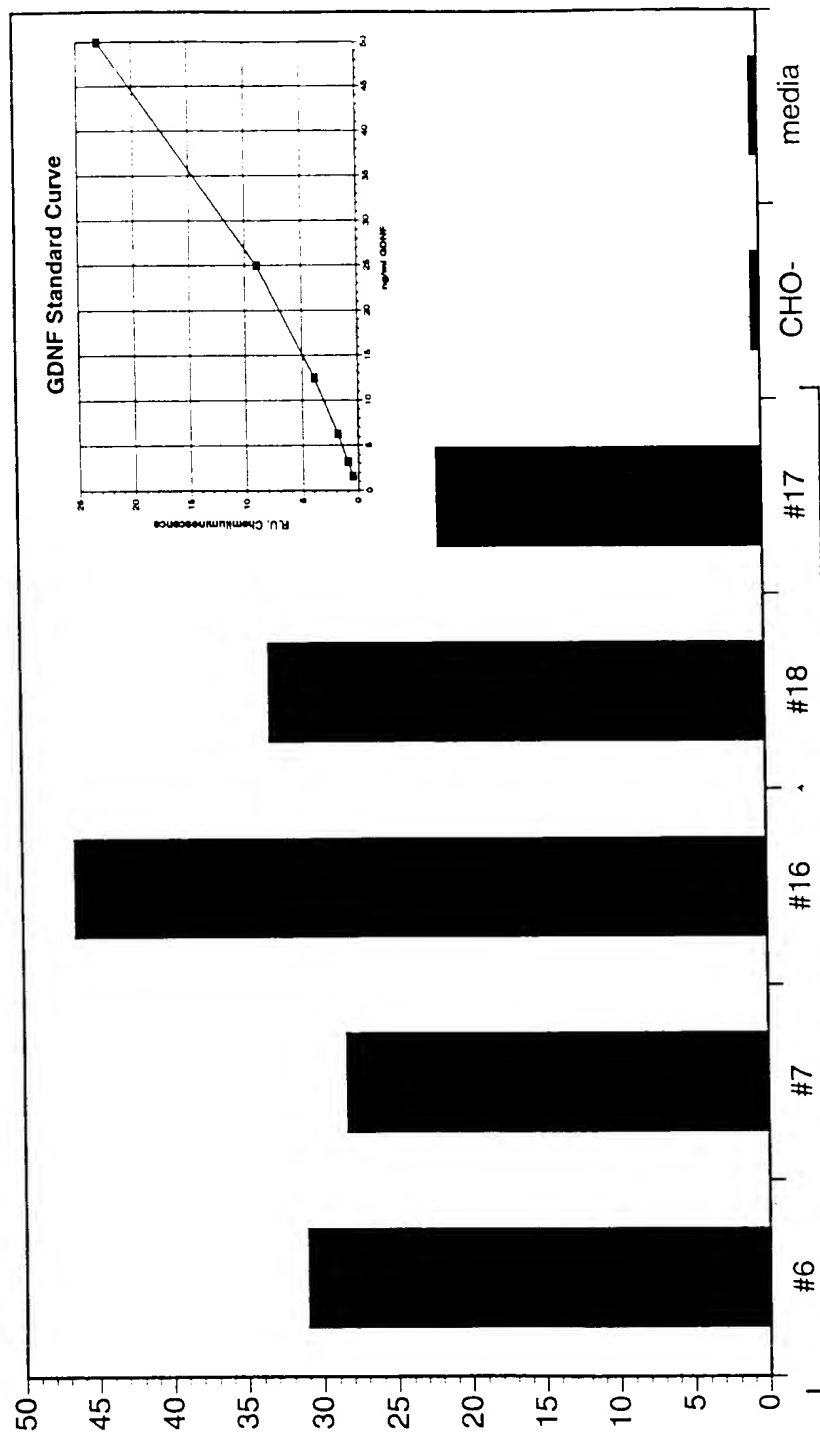


Fig. 9

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Estimated Neublabin Concentration [ng/ml]



CHO Neublabin Clones

Fig. 10

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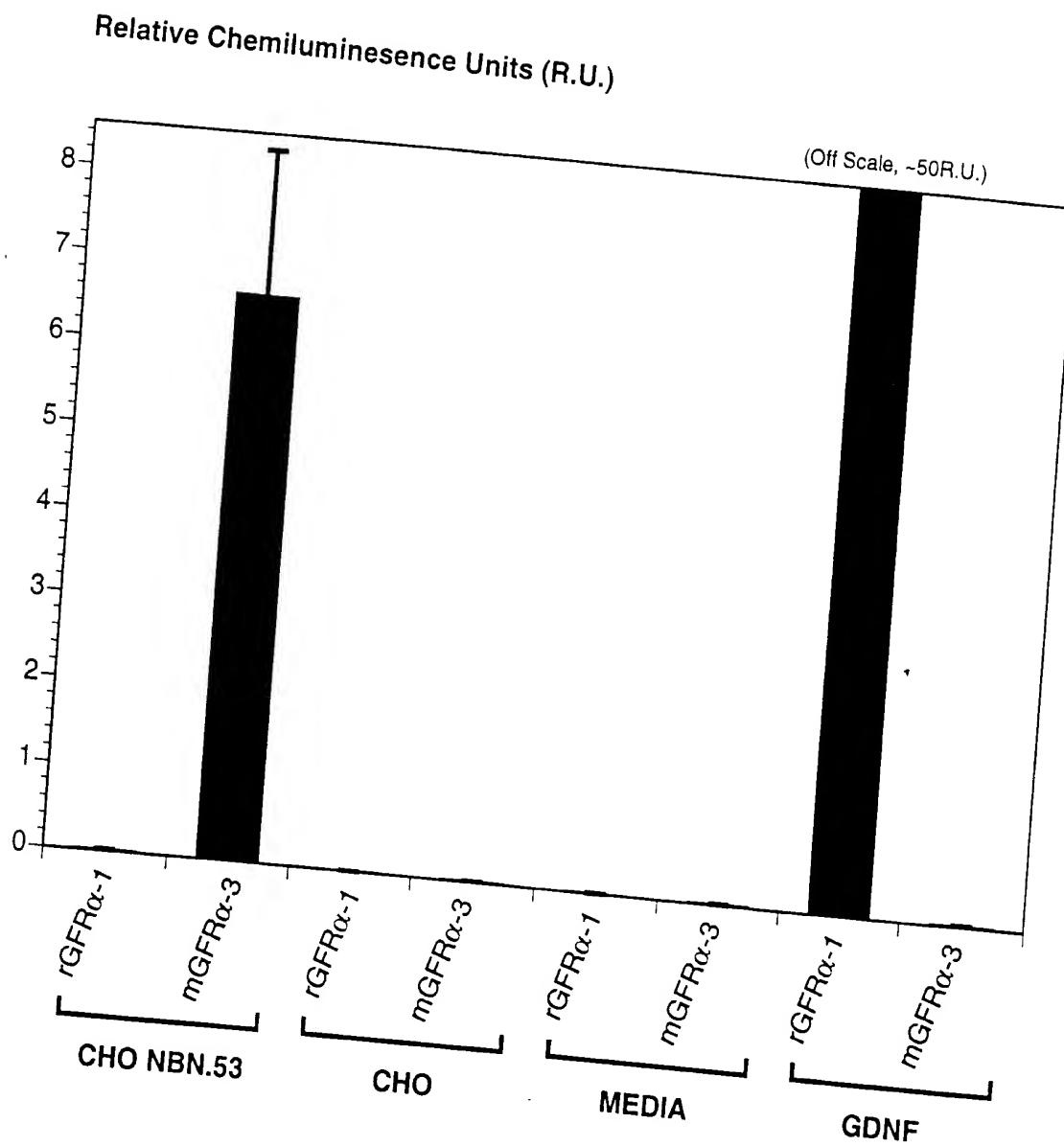
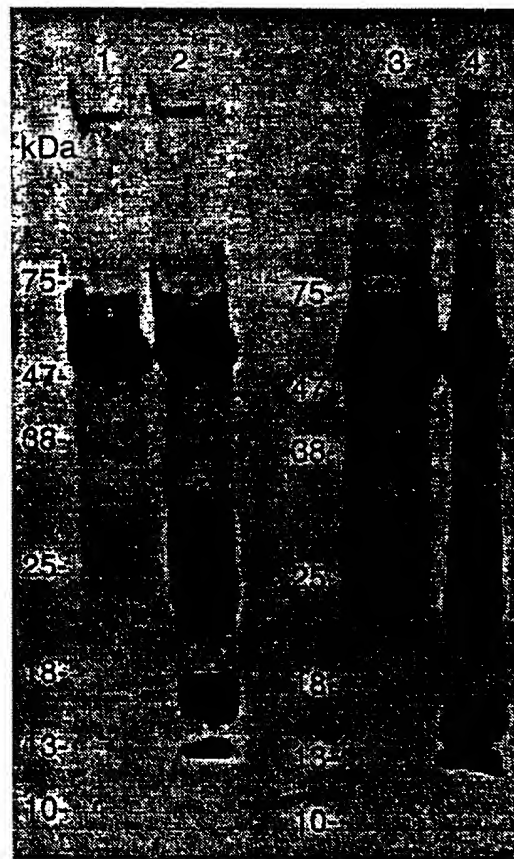


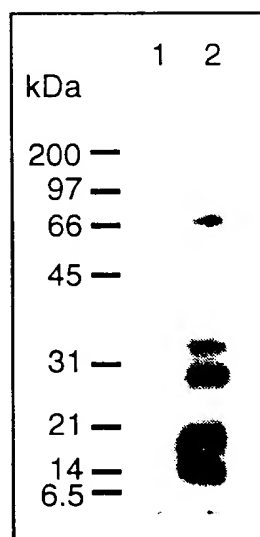
Fig. 11

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1. Control medium stained with R30 anti-peptide antibody
2. Neublastin containing conditioned medium stained with R30 anti-peptide antibody
3. Control medium stained with R31 anti-peptide antibody
4. Neublastin containing conditioned medium stained with R31 anti-peptide antibody

Fig. 12



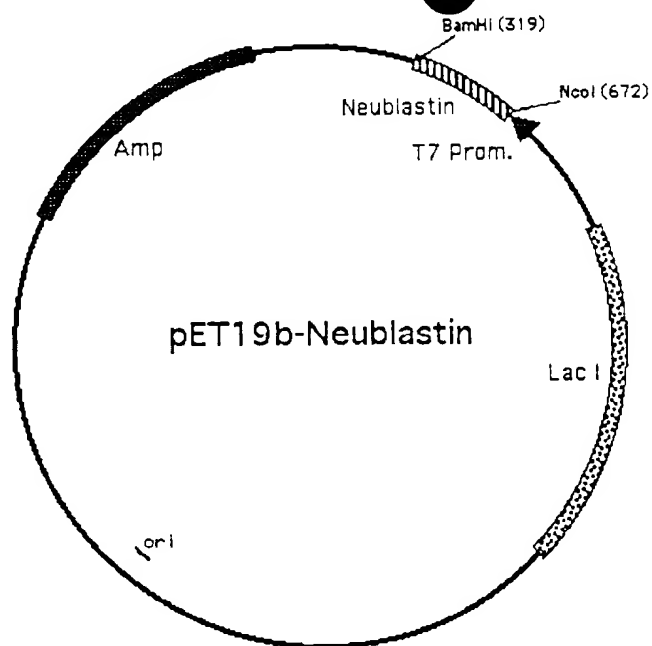
Extraction of neublastin by affinity-binding on RETL3-Ig

Lane 1: bound from CHO control conditioned media

Lane 2: bound from neublastin overexpressing CHO conditioned media

Fig. 13

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Neublastin Syngene

NcoI (318)

316 TACCATGGCT GGAGGACCGG GATCTCGTGC TCGTGCAGCA GGAGCACGTG GCTGTCGTCT
 ATCTTACCGA CCTCCTGGCC CTAGAGCACG AGCACGTCGT CCTCGTGCAC CGACAGCAGA
 1 ▶ M A G G P G S R A R A A G A R G C R L

376 GCGTTCTCAA CTAGTGCCGG TCGTGCACT CGGACTGGGA CACCGTTCCG ACGAACTAGT
 CGCAAGAGTT GATCACGGCC ACGCACGTGA GCCTGACCCT GTGGCAAGGC TGCTTGATCA
 19 ▶ R S Q L V P V R A L G L G H R S D E L V

436 ACGTTTTTCGT TTTTGTTTCAG GATCTTGTCG TCGTGACGT TCTCCGCATG ATCTATCTCT
 TGCAAAAGCA AAAACAAGTC CTAGAACAGC AGCACGTGCA AGAGGCGTAC TAGATAGAGA
 39 ▶ R F R F C S G S C R R A R S P H D L S L

496 AGCATCTCTA CTAGGAGCCG GAGCACTAAG ACCGCCGCCG GGATCTAGAC CTGTATCTCA
 TCGTAGAGAT GATCCTCGGC CTCGTGATTC TGGCGGCGGC CCTAGATCTG GACATAGAGT
 59 ▶ A S L L G A G A L R P P P G S R P V S Q

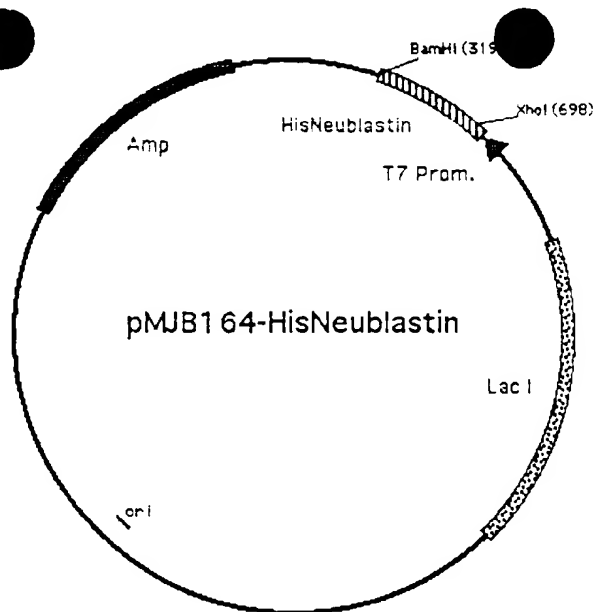
556 ACCTTGTTGT AGACCTACTA GATACGAAGC AGTATCTTTC ATGGACGTAA ACTCTACATG
 TGGAACAACA TCTGGATGAT CTATGCTTCG TCATAGAAAG TACCTGCATT TGAGATGTAC
 79 ▶ P C C R P T R Y E A V S F M D V N S T W

BamHI (671)

616 GAGAACCGTA GATAGACTAT CTGCAACCGC ATGTGGCTGT CTAGGATGAT AATAGGGATC
 CTCTTGGCAT CTATCTGATA GACGTTGGCG TACACCGACA GATCCTACTA TTATCCCTAG
 99 ▶ R T V D R L S A T A C G C L G . . .

676 CGGCT
 GCCGA

Fig. 14



HisNeubl原因

XhoI (340)

301 TACCATGGGC CATCATCATC ATCATCATCA TCATCATCAC TCGAGCGGCC ATATCGACGA
 ATCTTACCCG GTAGTAGTAG TAGTAGTAGT AGTAGTAGTG AGCTCGCCGG TATAGCTGCT
 1► M G H H H H H H H H S S G H I D D

361 CGACGACAAG GCTGGAGGAC CGGGATCTCG TGCTCGTGCA GCAGGAGCAC GTGGCTGTGC
 GCTGCTGTTT CGACCTCCTG GCCCTAGAGC ACGAGCACGT CGTCCTCGTG CACCGAÇAGC
 19► D D K A G G P G S R A R A A G A R G C R

421 TCTGCGTTCT CAACTAGTGC CGGTGCGTGC ACTCGGACTG GGACACCGTT CCGACGAACT
 AGACGCAAGA GTTGATCACG GCCACGCACG TGAGCCTGAC CCTGTGGCAA GGCTGCTTGA
 39► L R S Q L V P V R A L G L G H R S D E L

481 AGTACGTTTT CGTTTTTGTT CAGGATCTTG TCGTCGTGCA CGTTCTCCGC ATGATCTATC
 TCATGCAAAA GCAAAAACAA GTCCTAGAAC AGCAGCACGT GCAAGAGGCG TACTAGATAG
 59► V R F R F C S G S C R R A R S P H D L S

541 TCTAGCATCT CTA TAGGAG CCGGAGCACT AAGACCGCCG CCGGGATCTA GACCTGTATC
 AGATCGTAGA GATGATCCTC GGCCTCGTGA TTCTGGCGGC GGCCCTAGAT CTGGACATAG
 79► L A S L L G A G A L R P P P G S R P V S

601 TCAACCTTGT TG TAGACCTA C TAGATACGA AGCAGTATCT TTCATGGACG TAAACTCTAC
 AGTTGGAACA ACATCTGGAT GATCTATGCT TCGTCATAGA AAGTACCTGC ATTTGAGATG
 99► Q P C C R P T R Y E A V S F M D V N S T

BamHI (719)

661 ATGGAGAACC GTAGATAGAC TATCTGCAAC CGCATGTGGC TGTCTAGGAT GATAATAGGG
 TACCTCTTGG CATCTATCTG ATAGACGTTG GCGTACACCG ACAGATCCTA CTATTATCCC
 119► W R T V D R L S A T A C G C L G . .

721 ATCCGGCTGC TAACAAAGCC CG
 TAGGCCGACG ATTGTTTCGG GC

Fig. 15

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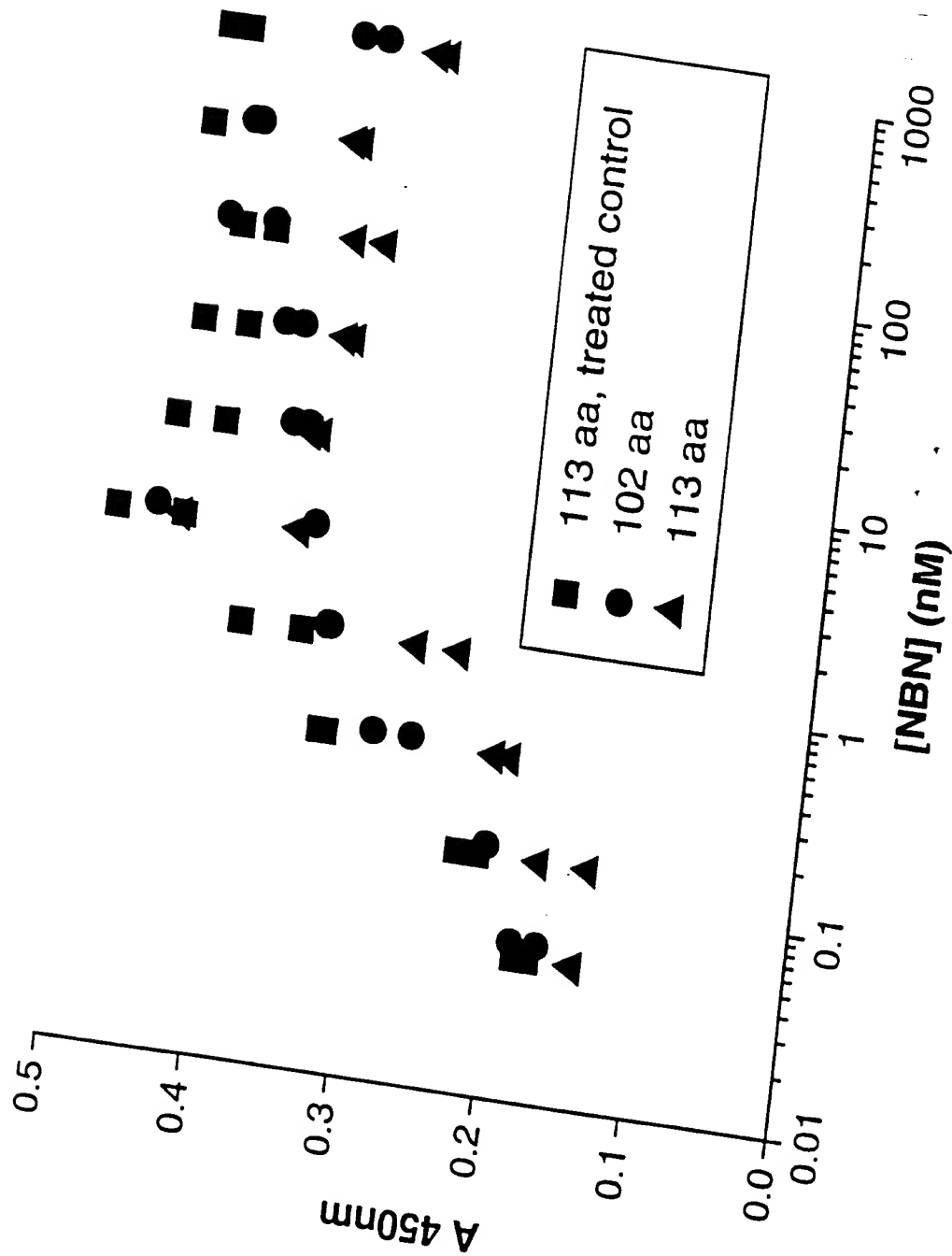
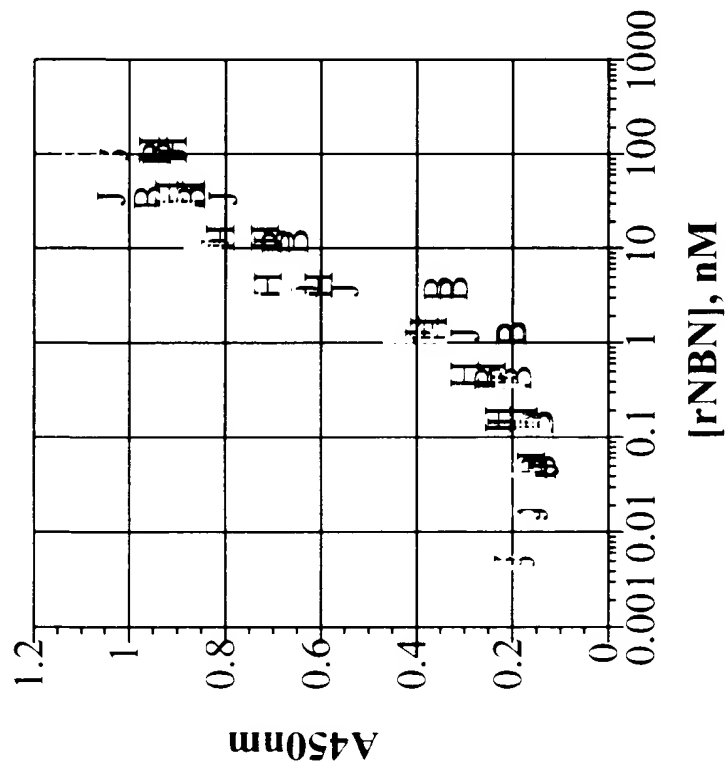


Fig 16

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B R14K (N) 0.15 mg/ml
 J R14K (N-14) 0.22 mg/ml LysC
 H R14K (N-7) 0.88 mg/ml
 WT 1.0 mg/ml (11-16-00 #4)

FIG. 17